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Research

Quinine sulfate and bacterial invasion

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Abstract

Background: As many patients who receive antimalarial drugs for treatment of noninfectious, inflammatory diseases are also immunosuppressed and might have a concomitant bacterial infection, we studied the effectiveness of these drugs against bacterial infections, to find out whether they could protect against (and even treat) such conditions and obviate the need for an additional antibiotic drug.

Methods: Effect of QS on bacterial growth: *Escherichia coli* (*E. coli*) HB101 pRI203 were cultured overnight at 37°C in TSB and inoculated (approx 1×10^7 cells /ml) in MEM in the presence of QS at various concentrations (0, 50 and 100 μM).

The effect of QS at concentration of 50 and 100 μM on the entry process of *E. coli* HB101 pRI203 into HeLa cells was studied under different experimental conditions: 1. QS was incubated with 3×10^5 HeLa cells for 60 min at 37°C prior to infection. 2. QS was added to HeLa cell monolayers during the infection period.

Results: QS showed no antibacterial activity after 24 h of incubation.

The invasive efficiency of the bacteria was significantly inhibited at a dose-dependent manner, when QS was added to HeLa cells for 60 min at 37°C prior to infection (condition 1), and to a lesser extent when added during the period of infection (condition 2).

Conclusions: Although the antimalarials are generally regarded as being inactive against most extracellular bacterial species, our results indicate that QS significantly inhibited the internalization/invasion efficacy of *E. coli* in the host cells.

Background

Antimalarial drugs were first discovered in the seventeenth century [1], two and one-half centuries before the causative agent of malaria was identified. As no other drug before it, quinine, the first antimalarial agent derived from the cinchona tree, helped to shape today's world by enabling explorers and colonists from Europe to survive in tropical countries and to build their colonial empires

despite lethal tropical malaria. When physicians were asked to choose the ten most important drugs being used in medicine in 1945, quinine and quinacrine were preceded only by penicillin, sulfonamides and blood derivatives [2]. The antimalarial activity of quinoline antimalarial drugs can be attributed to the interfering with hemoglobin digestion in the blood stages of the malaria parasite's life cycle. The parasite degrades the host hemoglobin, in an acidic food vacuole, to generate amino acids for its own protein synthesis, and producing free heme and reactive oxygen species as toxic by-products. The heme moieties are neutralized by polymerization, and this process is thought to be the biochemical target for antimalarial drugs. Although antimalarial drugs were developed primarily to treat malaria, and never lost their place in treating this life-threatening disease (still a major cause of infant death in the tropics [3]), they are also beneficial for many dermatological, immunological, and rheumatological diseases, for which they are mostly used today in the Western world [4].

Some of the many patients who receive antimalarials for the treatment of non-infective inflammatory diseases (lupus erythematosus and other collagen vascular diseases, vasculitis, panniculitis, rheumatoid arthritis and others) are also immunosuppressed because of their disease and/or treatments and have a concomitant bacterial infection. These patients often need systemic antibiotics, either as prophylaxis or for the treatment of active infections of the skin or other organs.

Therefore, if antimalarials could prove to be effective against bacterial infections, they can protect against (and even treat) such conditions and obviate the need for an additional antibiotic drug.

To examine this possibility, we studied the effect of quinine sulfate (QS) on bacterial growth and on bacterial invasion of cultured cells.

Methods

Organism and media

Escherichia coli (*E. coli*) HB101 (pRI203) was kindly provided by Dr. Falkow (Stanford Medical School, Calif. USA). The pRI203 plasmid carries a chromosomal DNA fragment of *Y. pseudotuberculosis* which converts the *E. coli* HB101 strain into an organism capable of invading cultured animal cells [5].

E. coli HB101 pRI203 were routinely cultured on trypticase soy broth (TSB; BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Ampicillin was added to the culture media at a final concentration of 50 μg/ml to select the maintenance of the recombinant invasive plasmid in all the experimental conditions.

Cells

HeLa S3 cells (epithelioid carcinoma of human cervix) were cultured in Minimum Essential Medium (MEM; Seromed) supplemented with 1.2 g/l NaHCO3, 2 mM glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 10% heat-inactivated fetal calf serum (FCS), in a 5% CO_2 incubator.

HeLa S3 cells were grown as monolayers at 37°C on 12-well tissue culture clusters (Costar) as described elsewhere [6].

Chemicals

Quinine sulfate (QS, M.W. 783 kDa) was dissolved in an ethanol-water ratio of 1:1.

Toxicity of QS towards cultured cells

Cells propagated in tissue-culture clusters were incubated with different concentration of QS (0, 50 and 100 μM) at 37°C for 2 h in MEM. The cell monolayers were then washed with phosphate-buffered saline (PBS); fresh medium was added and the cells were observed and stained after a 24 h incubation period at 37°C.

Effect of QS on bacterial growth

E. coli HB101 pRI203 were cultured overnight at 37°C in TSB and inoculated (approx 1×10^7 cells /ml) in MEM in the presence of QS at various concentrations (0, 50 and 100 μM). The determination of viable bacteria was performed after 24 h of contact by counting the colony-forming units (CFU) on trypticase soy agar (TSB, BBL).

Invasion assay

Invasion of cultured cells was assayed by a modification of the technique of Isberg and Falkow (1985) [7]. Briefly, semiconfluent monolayers of HeLa cells grown without antibiotics in 12-well plates were infected with bacterial suspensions (100 bacteria per cell) in the early exponential phase, corresponding to a subculture of 120 min at 37°C. Infection was performed for 1 h at 37°C. The cells were then thoroughly washed with PBS, and 1 ml of fresh medium containing 100 μg/ml gentamicin was added to each well. After a further 2-h incubation period at 37°C, infected cells were treated with trypsin-EDTA (mixture of 0.05 % trypsin [1/250] and 0.02 % EDTA) for 5 min at 37°C and lysed by the addition of 0.1 %Triton-X100. Cell lysates were diluted in PBS and plated on TSB to quantify the number of viable intra-cellular bacteria. The invasion was controlled by the microscopic observation of Giemsa-stained slides in all experiments.

Invasion assay in presence of QS

The effect of QS at concentration of 50 and 100 μM on the entry process of *E. coli* HB101 pRI203 into HeLa cells was studied under different experimental conditions:

Table 1: Invasion efficiency of *E. coli* HB101 pRI203 into HeLa cells in the presence of quinine sulfate (QS)

QS	condition 1	condition 2
0	2.95	2.95
50 μM	0.64	1.07
100 μM	0.46	1.18

Inoculated and internalized viable bacteria were evaluated by counting them on agar plates. Invasive efficiency is the percent of the inoculated colony-forming units (CFU) which were internalized. Condition 1: QS was incubated with 3×10^5 HeLa cells for 60 min at 37°C prior to infection; condition 2: QS was added to the HeLa cell monolayers during the infection period.

1) QS was incubated with 3×10^5 HeLa cells for 60 min at 37°C prior to infection; cell monolayers were then washed three times with PBS to remove the QS.

2) QS was added to HeLa cell monolayers during the infection period.

The invasive efficiency was expressed as the number of viable internalized bacteria by counting the CFU.

Results

The initial test of the toxicity of QS toward cultured cells indicated that after the incubation at 37°C for 2-h, QS at concentrations of 0, 50 and 100 μM, produce no damage to the HeLa cells.

To assay the viability of the microbial cells during the invasion experiments, different concentrations of QS 50 and 100 μM, were added to *E. coli* suspensions (1×10^7 CFU/ml) in MEM following an overnight culture at 37°C. QS showed no antibacterial activity after 24 h of incubation.

Effect of QS on E. coli HB 101 pRI203 invasion into cultured cells

HeLa confluent monolayers were infected with bacterial suspension in a logarithmic-phase growth for 60 min at 37°C in the presence and absence of 50 and 100 μM of QS. Extracellular bacteria were killed by the addition of gentamicin, and the intracellular bacterial CFU were counted on TSB after lysis of the infected monolayers. Table 1I displays the internalization of *E. coli* in the host cells, and is expressed as the percent of the inoculated colony-forming units which were internalized. The invasive efficiency of the bacteria was significantly inhibited, in a dose-dependent effect, by QS, when the drug was added for 60 min at 37°C prior to infection (condition 1). When the QS was added to HeLa cell monolayers during the infection period (condition 2), the invasive efficiency of the bacteria was also reduced but to a lesser degree than in the first condition.

Discussion

The antimalarials have recently been reported to be endowed with anti-HIV-1 activity and, therefore, proposed as additional therapy for HIV infection [8–12]. Their place in the treatment of autoimmune dermatological and rheumatological diseases has already been widely discussed in the literature [4]. Apart from their well-documented advantages, i.e., excellent bioavailability, low toxicity, long-term safety, and low-cost, one more property of these drugs might be of special interest in the setting of HIV infection and of autoimmune diseases, namely, their ability to prevent/treat several infectious diseases other than malaria. The antimalarials have been proven as being beneficial for several intracellular pathogens, but only intracellular ones that mainly affect individuals with impaired cell-mediated immunity, such as Legionella pneumophila, Histoplasma capsulatum, Francisella tularensis, Penicillium marneffei Cryptococcus neoformans, Bacillus subtilis, Mycobacterium avium, Mycobacterium tuberculosis, Toxoplasma gondii [13–20] and others.

Although the antimalarials are generally regarded as being inactive against most extracellular bacterial species, in the early seventies one British group reported that the growth of *E. coli* could be inhibited by chloroquine [21–24]. Another group found that antimalarials inhibited in vitro the activity of DNA polymerase and RNA polymerase of *E. coli*[25], thus confirming findings of earlier experiments by others [26,27].

The adhesion of *E. coli* to host epithelial cells is the very first step of infections, followed by the internalization of some bacteria into the cells. The internalization of attached bacteria is a kind of endocytosis rather than an invasion and is thus influenced by the adherence of bacteria to the epithelial cells. According to Straube et al. chloroquine leads to a significant inhibition of internalization of bacteria by inhibiting endocytosis [28].

In contrast to previous findings [21–24] by Wiseman's group (which were carried out on chloroquine and not quinine), our QS experiments failed to show any antibacterial activity or any effect on bacterial growth what-so-ever.

Our in vitro invasion assays, however, did show that QS significantly inhibited the internalization/invasion efficacy of *E. coli* in the host cells, in accordance with one previous study [28].

Conclusions

Our data further support the finding that QS interferes with invasion and internalization of *E. coli* into host cells, thus protecting living cells from its pathogenic effects. In contrast to the results of previous reports, we demonstrated that it does not affect bacterial growth.

Apart from the theoretical considerations of these findings in providing further insight into the pathomechanism of bacterial invasion and the contra-action of antimalarial drugs, our current findings might also have practical implications in illuminating the antibacterial action of antimalarial drugs, data which might be especially important to immunosuppressed patients who receive these drugs for autoimmune collagen vascular diseases, or as additional therapy for AIDS. It is worth noting that the antimalarials do not act directly on the invading pathogens, but rather on the host cells and, therefore, the potential for microorganisms to become resistant to its effects may be limited.

The antibacterial action of antimalarials against various other common bacteria is currently under investigation by our group.

Authors' contributions

Ronni Wolf, Eleonora Ruocco and Vincenzo Ruocco conceived the idea, designed of the study, drafted the article and revised it critically for important intellectual content. Maria Antonietta Tufano supervised all laboratory measurements and compilation of results, wrote the material and methods and results section of the first drafts and did the final critical analysis. Adone Baroni, Rita Greco, and Giovanna Donnarumma participated in design and coordination and carried out the experimental work. All authors read and approved the final manuscript.

List of abbreviations

QS: Quinine sulfate

E. Coli: *Escherichia coli*

TSB;BBL: Trypticase soy broth; BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA

MEM: Minimum Essential Medium

FCS: Fetal calf serum

PBS: Phosphate-buffered saline

CFU: Colony-forming units

HIV: Human immunodeficiency virus

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